

IT IS CLAIMED:

1. A microfabricated device for sorting reporter-labelled polynucleotide molecules by size, comprising

5 a chip having a substrate into which is microfabricated at least one analysis unit, said analysis unit comprising

a main channel, having at one end a sample inlet, having along its length a detection region, and having, 10 adjacent and downstream of said detection region, a branch point discrimination region;

a plurality of branch channels originating at the discrimination region and in communication with the main channel;

15 means for passing a continuous stream of solution containing said molecules through said detection region, such that on average only one molecule occupies the detection region at any given time;

means for measuring the level of reporter from each 20 molecule within the detection region; and

means for directing said molecule to a selected branch channel based on said level of reporter.

2. The device of claim 1, wherein said directing 25 means comprises a pair of electrodes effective to apply an electric field across the discrimination region, said field being effective to direct a particular molecule into a selected branch channel.

30 3. The device of claim 1, wherein a flow of molecules is maintained through the device via a pump or pressure differential, and said directing means comprises a valve structure at said branch point effective to

permit said molecule to enter only one of said branch channels.

4. The device of claim 1, wherein a flow of
5 molecules is maintained through the device via a pump or pressure differential, and said directing means comprises, for each branch channel, a valve structure downstream of said branch point effective to allow or curtail flow through said channel.

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5. The device of claim 1, wherein a flow of
molecules is maintained through the device via a pump or pressure differential, and said directing means comprises, for each branch channel, a pressure adjusting means
15 at the outlet of each branch channel effective to allow or curtail flow through said channel.

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6. The device of claim 1, wherein said channels are between about 1 and 10 μm in width and between about 1 and 10 μm in depth.

7. The device of claim 1, wherein said detection region has a volume of between about 1 fl and 1 pl.

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8. The device of claim 1, further comprising a glass cover slip bonded to said substrate and covering said channels.

9. The device of claim 1, wherein said exciting and
30 measuring means comprises a fluorescence microscope holding said device.

10. The device of claim 1, wherein said exciting means comprises an external or integrated semiconductor laser, and said measuring means comprises an integrated photodiode.

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11. A method of isolating polynucleotides having a selected size, comprising

10 A) flowing a continuous stream of solution containing reporter-labeled polynucleotides through a channel comprising a detection region having a selected volume, where the concentration of the molecules in the solution is such that most molecules pass through the detection region one by one,

15 B) determining the size of each molecule as it passes through the detection region by measuring the level of said reporter,

20 C) in said continuous stream of solution, diverting (i) molecules having said selected size into a first branch channel, and (ii) molecules not having said selected size into a second branch channel, and

25 D) collecting polynucleotides diverted into said first branch channel.

12. The method of claim 11, wherein the concentration of polynucleotides in said solution is between about 100 fM and about 100 pM.

30 13. The method of claim 11, wherein said detection region volume is between about 1 fl and about 1 pl.

14. The method of claim 11, wherein said determining includes quantitating an optical signal from an optical reporter associated with said polynucleotides.

5 15. The method of claim 14, wherein said optical signal from an optical signal is a fluorescence signal and said optical reporter includes a fluorescent moiety.

10 16. The method of claim 15, wherein said optical reporter is selected from the group consisting of POPO, BOBO, YOYO, and TOTO.

15 17. The method of claim 11, wherein said diverting includes the transient application of an electric field effective to bias (i) a molecule having said selected size to enter said first branch channel, and (ii) a molecule not having said selected size to enter said second branch channel.

20 18. The method of claim 11, for diverting a molecule having said selected size into said first branch channel, wherein said diverting includes blocking the flow in said second branch channel such that said continuous stream of solution carries the molecule having
25 said selected size into said first branch channel.

30 19. The method of claim 11, for diverting a molecule not having said selected size into said second branch channel, wherein said diverting includes blocking the flow in said first branch channel such that said continuous stream of solution carries the fragment not having said selected size into said second branch channel.

20. The method of claim 11, wherein said diverting includes a mechanical switch effective to direct (i) a fragment having said selected size to enter said first branch channel, and (ii) a fragment not having said
5 selected size to enter said second branch channel.

21. The method of claim 11, wherein said selected size is between about 100 bp and about 10 mb.

10 22. The method of claim 21, wherein said selected size is between about 100 bp and about 100 kb.

23. The method of claim 22, wherein said selected size is between about 500 bp and about 50 kb.

15 24. A method of sizing polynucleotides in solution, comprising

20 A) flowing a continuous stream of solution containing reporter-labeled polynucleotides through a microfabricated channel comprising a detection region having a selected volume, where the concentration of the molecules in the solution is such that most molecules pass through the detection region one by one, and

25 B) determining the size of each molecule as it passes through the detection region by measuring the level of said reporter.